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Alternative Filtration Methods for Removal of 'Giardia' Cysts and Cyst Models

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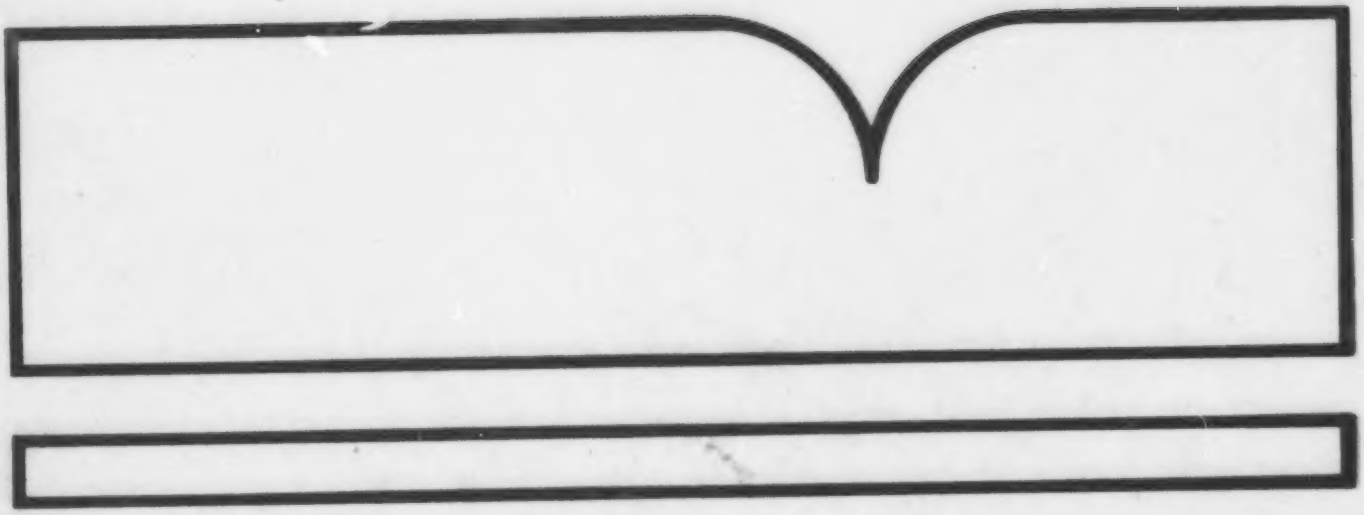

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16. ABSTRACT

Recent occurrence of numerous outbreaks of waterborne giardiasis has renewed interest in water filtration for cyst removal.

This paper presents an in-depth review of granular media and diatomaceous earth (D.E.) filtration research conducted in the 1930's and 1940's when Entamoeba histolytica cyst problems were a great concern.

Recent studies with cyst models and Giardia muris cysts suggest that properly operated water filtration plants should be able to remove a high percentage of G. lamblia cysts. To do so, D.E. filters need 1.0 kg/m^2 diatomite precoat plus body feed. The septum must be kept clean also. Effective cyst removal by granular media filters requires coagulation of raw water and production of filtered water with turbidity well below 1.0 NTU throughout the entire filter run. Abrupt increases in filtration rate should be avoided.

17. KEY WORDS AND DOCUMENT ANALYSIS

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tions. In fact, it was unequivocally established within the first full year of operations that total elimination of objectionable odors and tastes—the primary objective of using ozonation in this case—can be achieved effectively, reliably, and economically under all conditions.

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Alternative filtration methods for removal of *Giardia* cysts and cyst models

Gary S. Logsdon, James M. Symons, Robert L. Hoye Jr., and Michael M. Arozarena

The recent occurrence of waterborne giardiasis outbreaks has spurred renewed interest in water filtration for cyst removal. Studies with cyst models and *Giardia muris* cysts suggest that properly operated water filtration plants should be able to remove a high percentage of *G. lamblia* cysts. For effective cyst removal, diatomaceous earth (DE) filters need 1.0 kg/m² diatomite precoat plus body feed, and granular media filters require coagulation of raw water and production of filtered turbidity well below 1.0 ntu throughout the entire filter run.

Outbreaks of waterborne giardiasis, a disease caused by a protozoan parasite, have been reported more frequently in recent years. This trend is a cause for concern among public health, environmental, and water utility officials. Because of the need for information on the efficacy of water treatment for *Giardia* cyst removal, a research program on this topic was undertaken by the US Environmental Protection Agency's drinking water research division. Objectives of the study included learning whether or not a relationship existed between cyst removal or cyst concentration in filtered water and the turbidity of filtered water, identifying treatment procedures and operational problems that might allow cysts to pass through filters, and discovering the cyst removal capabilities of well-operated filters.

Literature review

A review of literature revealed no research on water filtration for *Giardia* cyst removal. This is not surprising because until recently the *Giardia* organism was not implicated as a cause of waterborne disease. The hazards of waterborne amoebiasis, however, have been known for decades. Water filtration studies for removal of *Entamoeba histolytica* cysts were carried out in the 1930s and 1940s.

Granular media filtration research. After the Chicago amoebiasis outbreak occurred in 1933, experiments for removal of *E. histolytica* cysts from water were carried out at the experimental filtration plant operated by the Chicago Department of Public Works. In preliminary laboratory experiments, 4-cm diameter filter columns were used. Tests with these small columns showed complete removal of *E. histolytica* cysts from water by coagulation and rapid sand filtration.[1]

Further testing with a pilot plant filter having a surface area of 0.9 m² took place in 1935 and 1936.[2] Cysts were added to previously coagulated water that was then treated by direct filtration at a rate of 5 m/h. Samples were taken at regular intervals during the filter runs, which ranged in duration from 4 to 14 hours. The samples were concentrated in a continuous centrifuge, and the sediment was examined in a counting cell commonly used for algae counting.

On the basis of the total volume of water filtered and the total number of cysts applied during the filtration experiments, removal efficiency exceeded 99.99 percent. Baylis et al[2] concluded that filtration of water through rapid sand filters, in the manner then extensively used in the United States (5 m/h [2 gpm/sq ft]), was effective in removing *E. histolytica* cysts from water.

During World War II the need to provide water free of *E. histolytica* cysts resulted in a cooperative filtration research program involving the Engineer Board of the US Army at Ft. Belvoir, Va., and the National Institute of Health, US Public Health Service. Although a report of this work was prepared by the War Department,[3] it was not widely circulated. This paper presents a summary of that report, subsequently referred to as the Army report.

Granular media filtration tests were performed with a 43-cm inside diameter pressure filter vessel that held sand filter media 46-cm deep, with an effective size of 0.36 mm and a uniformity coefficient of 1.35. Media support was provided by 10 cm of fine gravel.

Filtration tests were performed with and without coagulation, and with and without sedimentation. Results of these tests, summarized in Tables 1 and 2, show the influence of good operating practice on cyst removal by granular media filters. Tests 1, 6A, and 6B involved filtration of uncoagulated water; test 2 was performed with poorly coagulated water. For these tests, the numbers of cysts passing the filter per

TABLE 1

Army portable water purification unit (sand filter).
Summary of the results of E. histolytica experiments under several operating conditions[3]

Experiment Number	Quality of Coagulation	Filtration Rate m/h	Calculated Number of Cysts per Litre		Number of Cysts Passing Filter per Million Applied	Reduction percent
			Influent	Effluent		
1	none	23	550	66	119 000	88.1
2	poor	23	1700	63	38 000	96.2
3	good	23	610	2.4	3900	99.6
4	good	15	1300	1.2	900	99.9
5	good	15	1800	2.6	1400	99.86
6B	none	15	220	44	197 500	80.3

TABLE 2

Army portable water purification unit (sand filter).
Summary of the results of E. histolytica experiments with operation at reduced rate following coagulation and sedimentation[3]

Experiment Number	Pretreatment	Settling Time min.	Filtration Rate m/h	Calculated Number of Cysts per Litre		Number of Cysts Passing Filter per Million Applied	Reduction percent
				Influent	Effluent		
6A	none	90		2600	220	83 800	91.62
6B			15	220	44	197 500	80.25
Combined results						16 500	98.35
7A	alum and soda ash	120		1000	1.4	1400	99.86
7B			15	1.4	0.2	176 000	82.4
Combined results						250	99.975
8A	alum and soda ash	75		1000	15	14 700	98.53
8B			15	15	0.5	35 700	96.43
Combined results						526	99.947

TABLE 3

Diatomaceous earth filters: Summary of operating conditions and E. histolytica cyst data[3]

Test	Precoat kg/m²	Body Feed mg/L	Flow Rate Range		Volume Filtered L	Volume Analyzed L	Cyst Data		Recovered Applied
			L/s	m/h			Dosed/L	Total Recovered	
1D	0.80	0	0.0063–0.0013	1.9–0.4	4	4	87 500	0	0/350 000
2D	0.50	0	0.088–0.0038		57	23	1565	3	3/36 000
4D	0.50	0				42	2620	1	1/110 000
5D	0.75	0		5.10		38	1580	0	0/60 000
6D	1.8				5.1	5.1	2640–3170	0	0/15 000
7D	0.85	170	1.4–0.8	6.2–3.4	2635	64	1195	7	7/76 500
8D	0.75	120	1.5–1.1	6.8–6.1	1836	23	940	1	1/21 600
9D	0.75	120	1.8–1.5	10.1–8.6	2453	23	505	0	0/11 610
10D	0.75	0	1.5–0.9	8.6–5.0				1	
11D	0.75	0	1.1–0.8	6.5–4.3				0	
12D	0.75	0	1.6–0.8	9.1–4.8				1	
13D	0.75	0	1.8–1.1	10.6–6.5				0	

NOTE: Authors of the Army report did not publish complete data on tests 10D to 13D, but they did make the following comments:

"j. Experiments 10D through 13D inclusive; US Army portable water purification unit, model 1940, converted for use with diatomaceous silica. . .

The purpose of this series of tests was to determine the effectiveness of various grades of diatomaceous silica in the removal of cysts from water. Attention is directed to the absence of cysts in quantity in the effluent from all of the materials tested. Since cysts were removed by all of the materials tested, the significant feature of these tests is that no cysts passed the unit in experiment 13D. The filter aid used in the experiment was Johns-Manville Celite 545, which is too coarse for water filtration. Notwithstanding the passage of turbidity in excess of 10 ppm no cysts were found in the effluent samples . . . It appears, therefore, that any currently manufactured filter-aid in the range of the materials tested will produce satisfactory results."

million applied were much higher than for other tests with good coagulation and comparable experimental conditions. Filtration rates were 15 or 23 m/h for the Army tests.

Diatomite filtration. In the United States the Army took the lead in developing diatomaceous earth (DE) filtration for drinking water with a research and development program during World War II.[3] A discussion of DE filtration principles and a summary of bacteriological data developed in the project were also published by Black and Spaulding,[4] but the paper merely mentioned a need to remove cysts of *E. histolytica*, without giving actual test results. Experimental data are given in the Army report.

Experiments on diatomaceous earth filtration for cyst removal were carried out with a variety of DE filter systems, ranging from devices providing batch filtration of as little as 4 L of water to equipment capable of filtering approximately 75 L/min. Table 3 summarizes operating conditions and *E. histolytica* data for DE filters.

Newly calculated data include all data for the ratios of cysts recovered from filtered effluent to cysts in the same volume of filter influent, as well as some data on filter aid usage and flow rates. Some data were not available in the Army report so no values are given in certain instances in Table 3.

Data in Table 3 support the Army's conclusion that DE filtration could remove essentially all cysts. The largest number of cysts recovered, seven, resulted from using contaminated water (225 000 cysts in 130 L) to precoat the filter. Clean, filtered water should always be used for filter precoating.

Giardia-related research methods

Past research on cyst removal was related to treatment for *E. histolytica* cysts. Research results demonstrated that these cysts could be removed and suggested that filtration should also be effective for reducing the concentration of *Giardia* cysts in water. To demonstrate this specifically, a program of filtration research with *Giardia* cysts and cyst models was conducted at EPA's environmental research center. Both granular media and DE filters were studied. Certain aspects of the work, such as preparation of cyst suspensions and microscopic examination of samples, were common to both kinds of filtration research. They are presented first.

Use of cyst models. When the DE filtration research was begun in 1977, the difficulty of detecting and counting *Giardia* cysts in water necessitated a model for the cysts. Radioactive, 9-μm diameter microspheres* were used because they were very similar in size to *Giardia* cysts and were easy to trace. The resin bead microspheres were coated with cerium-141 to prevent the isotope from leaching. The radioactivity of a single microsphere could be differentiated from background radiation in the low-level scintillation counter that was used to measure gamma radioactivity on the 5-μm pore diameter membrane sampling filters.

The first phase of diatomaceous earth filtration research was performed with the microspheres. Because DE filters remove particles in the *Giardia* size range by a straining process, the radioactive particles were useful models for the cysts. The ease and sensitivity with which the microspheres were detected facilitated performing a large number of tests related to the effects of various aspects of filter operation on passage of

*NEN-TRAC, New England Nuclear, Boston, Mass.

cyst-sized particles through the DE filter. Subsequent studies with *G. muris* cysts confirmed the efficacy of DE filtration for cyst removal.

Use of *Giardia* cysts. Because it was difficult to obtain a constant source of viable *G. lamblia* cysts for research, mice were infected with *Giardia muris* and used as a source of cysts. Nearly all of the filtration research was done with the radioactive beads or with *G. muris* cysts, rather than with *G. lamblia* cysts.

G. muris should be a suitable surrogate for *G. lamblia* in filtration studies because both species are similar in size, shape, and surface electrical charge. EPA analysis* showed that *G. muris* cysts in distilled water had a zeta potential of −26 mV at pH 5.5. This figure agrees well with research conducted at the University of Washington,[5] where different equipment† gave a *G. lamblia* zeta potential of about −25 mV at pH 5.5. The *G. lamblia* cysts showed increased electronegativity at pH 8. Some physical characteristics of *E. histolytica* cysts and *Giardia* cysts and beads are given in Table 4.

Cyst preparation and counting. Certain laboratory techniques for handling the cysts, such as removal and concentration from fecal samples and cyst counting, were common to all of the research. Others, such as dosing the raw water and separating the cysts from filtered water, varied from time to time.

Cysts were obtained from mouse feces, and the suspensions were cleaned by a sucrose discontinuous gradient technique.[8,9] The washed cyst suspensions were refrigerated in "normal" saline solution until used.

Cyst enumeration by the hemocytometer method. The sample in the hemocytometer was examined under a light microscope, and cysts were identified on the basis of their morphology. Because examination of even 1 or 2 mL of water in the hemocytometer would be very time-consuming, only a fraction of the filtered water could be examined. As a result, a variable lower limit of detection always existed, and the finding of no cysts in the hemocytometer never could be interpreted as representing a cyst concentration of 0 cysts/L.

Cyst recovery from samples. Cysts were separated from the filtered water by use of 5-μm pore size polycarbonate filters. Effluents from the DE filtration research tests were passed through a 142-mm diameter membrane. After termination of the filter run the cysts were removed from the membrane by repeated washings with 0.85 percent saline. The washings were concentrated by centrifugation, the supernatant liquor was aspirated off, the concentrate was vortex-mixed, and a small, measured amount was counted in the hemocytometer.

In granular media filtration tests, water samples were periodically collected in 1-L cubitainers for refrigeration until they could be filtered through 47-mm diameter polycarbonate filters having 5-μm diameter pores. The polycarbonate filters were repeatedly washed with 0.85 percent saline, and the analysis continued as described above.

In order to evaluate the loss of cysts to DE filter apparatus and the failure of cysts to be recovered from the 142-mm membrane filter, tests were performed in which $7\text{–}21 \times 10^6$ cysts were instantaneously dosed to the filter when it was being operated with an uncoated septum. When the filter was operated at 3.8 L/min, four recovery tests averaged 10.3 percent recovery with a standard deviation of 2.7 percent. Therefore, during DE filtration experiments at 3.8 L/min the number of cysts recovered was corrected by dividing by 0.10. Later, two tests at 5.3 and 6.6 L/min showed 40 and 45 percent recovery, considerably higher than 10 percent. The higher velocities caused by greater flow rates may have reduced the tendency of the cysts to adhere to the DE filter apparatus. Nevertheless, to provide a conservative interpretation of the results of the higher rate tests, cysts recovered were divided by 0.10 rather than by 0.40 to 0.45 to estimate cyst removal percentages. This would tend to underestimate the percentage somewhat and yield conservative recovery rates.

The recovery efficiency of the 47-mm diameter polycarbonate filters used in the granular media study was determined by measuring cyst recovery from six separate 1000-mL samples, each of which had been dosed with about 3.5×10^6 cysts. Cyst recovery ranged from 55 to 65 percent for an average of 61 percent. For granular media filtration research, recovered cysts were divided by 0.61 to account for the efficiency of cyst recovery.

The water used for this research was obtained from an inactive gravel pit. This surface water was selected because of its clarity (less than 5 ntu) year-round. With respect to turbidity, this water resembles those where giardiasis outbreaks have occurred.

Diatomaceous earth filtration studies

The drinking water research division's pilot plant facilities included a 0.1-m^2 DE filter,[10] which was used in treatment studies with *Giardia* cysts and radioactive bead cyst models. The test results, presented more fully elsewhere,[11] are briefly summarized here.

Experimental methods. The DE test filter was set up for operation as a pressure filter with a slurry feeder for body feed. The 0.1-m^2 filter was operated at 2.4 or 3.5-m/h rates. The filter system is shown in Figure 1. Operation of the DE filter consisted of three steps—precoating, filtration, and backwashing.

A diatomaceous earth slurry was prepared by adding the desired weight of DE to tap water in the precoat tank and mixing the contents of the tank during precoating. The weight of diatomite added to the precoat tank was used to calculate precoat thickness expressed as kg/m^2. The slurry was recirculated until the water in the precoat tank appeared clear and free of diatomite.

During the filtration process a small amount of diatomaceous earth body feed was continuously added to the raw water in order to maintain good hydraulic characteristics of the precoat filter cake. Without the body feed, clay, bacteria, and other particulates found in natural waters and removed by the filter would soon coat the surface of the filter cake. The pores would become plugged, and a pressure drop would occur in the filter.

When body feed is added, the depth of the filter cake increases during the run and the major constituent of the filter cake is DE rather than removed particulates. The result is a porous filter media with good flow characteristics. In order to properly adjust body feed dose, some DE filter plant operators add body feed in proportion to raw water particulates. Turbidity is used as an indicator of the particulate content of water, so a body feed to turbidity (mg/L to ntu) ratio is sometimes used as a guideline for the needed dose of body feed. An economical body feed to turbidity ratio would vary for different raw waters. Body feed to turbidity ratios in this work generally ranged from 2:1 to 35:1.

A diatomaceous earth filter run generally is terminated because of head loss considerations. Because the filter cake increases in thickness during the run, filtered water turbidity should not increase rapidly (turbidity breakthrough) as with granular media filters. Turbidity breakthrough can occur, however, if the flow of water through the filter is interrupted, because interrupting the flow can cause the diatomaceous earth cake to fall off the filter septum. This should not be allowed to occur.

When a DE filter run is completed, the cake is removed from the septum, spent diatomite is discharged, the septum is cleaned, and the filter is made ready for precoating again.

An important feature of the filter system was the membrane sampling filter located downstream from the DE filter. The purpose of this membrane filter was to sample the entire effluent from the test filter and thus attain more reliable data on removal of cysts and cyst models.

When radioactive microspheres were used, the total number of microspheres passing the DE filter in a run could be

*Laser zee meter, PenKem, Inc., Croton-on-Hudson, N.Y.
†Zetameter, Zeta Meter, Inc., New York, N.Y.

determined directly by counting the membrane filter in a low-level gamma counter. The ability to identify a single bead in the total effluent for a filter run meant that the passage of one 9-μm radioactive bead could be detected. The microspheres were dosed on a continuous basis throughout the runs. Filter runs with microspheres ranged in duration from 2 to 27 hours with a typical run time of 6 to 8 hours.

When *G. muris* cysts were used in DE filtration tests, they were dosed in one massive slug, generally of 20–40 × 10^6 cysts, just before the raw water entered the pressure filter vessel. Filter operation continued long enough for at least 99 percent of the cysts to pass through the filter, as calculated by a first order differential equation for complete mixing within the filter vessel. Usually this operation ran 20 or 30 min. After the filter run, the 142-mm diameter membrane filter was carefully removed from its holder. The filter was washed and cysts were concentrated and counted as described in the section on cyst enumeration.

Results and discussion. Results of tests with radioactive beads (Figure 2) indicated that in most cases reduction of microsphere concentrations exceeded 99.9 percent. Filter performance was not related to turbidity reduction, a finding very similar to that in the Army report.[3] A probable explanation for this is that the cysts were removed even though clays and other particulates that caused turbidity in the gravel pit water were small enough to pass through the filter. The smallest cyst dimension would be about 7 μm, whereas clays and bacteria would be as small as 1 μm and thus more likely to pass through the pores of the filter cake. Therefore, performance is related to good operating procedures and not necessarily to turbidity removal.

Data obtained from the filter runs in which microspheres were used are plotted to show the differences in filter performance that are related to filter operating procedures. Specifically, reduction of microsphere concentration met or exceeded 99.9 percent in 21 tests when the precoat applied was at least 1.0 kg of diatomite per m^2 of filter surface and when body feed was utilized. Reduction of the microsphere concentration was less than 99.9 percent in only two tests with these operating conditions.

Filter operation with a precoat thickness of less than 1.0 kg/m^2 (two runs), with no body feed (three runs), or with less than 1.0 kg/m^2 of precoat as well as no body feed (three runs) resulted in removal of less than 99.9 percent of the microspheres. The test results show the importance of properly establishing the filter cake by using an adequate amount of precoat diatomite and of maintaining the integrity of the cake during a filter run by applying body feed.

TABLE 4
Physical characteristics of beads and cysts

Parameter	*E. histolytica*[7]	*G. lamblia*[8]	*G. muris*[8]	Microsphere
Shape	usually spherical	ovoid to ellipsoidal	ovoid to ellipsoidal	spherical
Dimensions	10–20 μm	8–12 × 7–10 μm	7–13 × 5–10 μm	9 μm
Zeta potential at pH 5 to pH 8	unknown	electronegative	electronegative	not measured

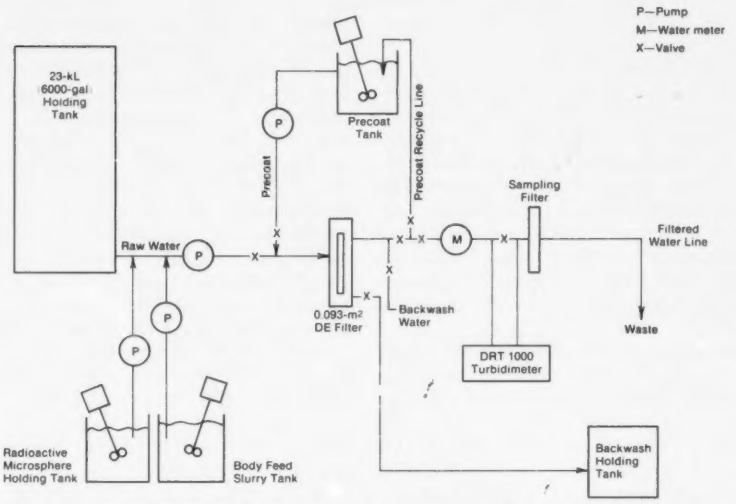

Figure 1. Schematic diagram of a diatomaceous earth (DE) pilot plant filter system

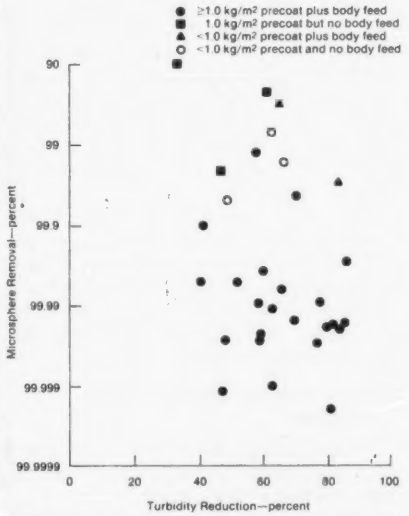

Figure 2. Relationship of water quality and DE filter operation

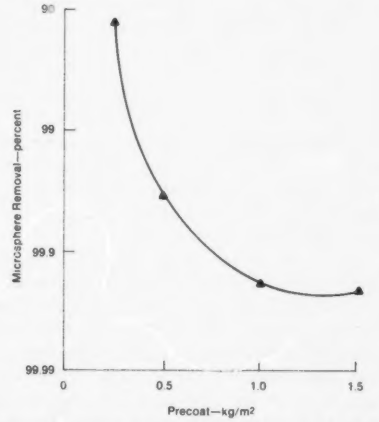

Figure 3. Effect of precoat amount on microsphere removal

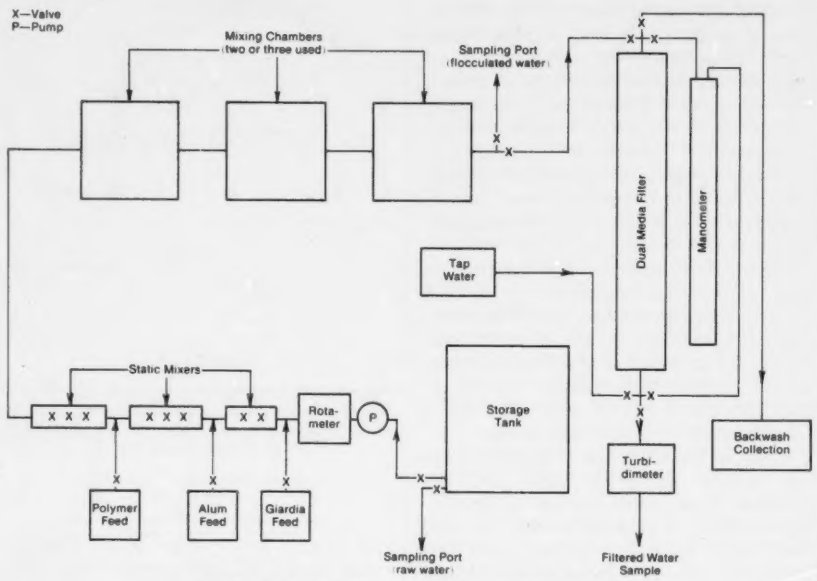

Figure 4. Schematic of granular media filtration

TABLE 5
*Diatomaceous earth filtration for G. muris cyst removal**

Filtration Rate m/h	Turbidity: Raw Water ntu	Turbidity: Filtered Water ntu	Body Feed mg/L	Cysts Dosed	Cysts Recovered	Reduction percent
2.3	2.5	0.58	31	22.0×10^6	2.8×10^4	99.87
2.4	2.5	0.56	30	18.7×10^6	3.3×10^4	99.82
2.2		0.76	33	24.4×10^6	1.56×10^3	99.36
2.4		0.49	15	19.2×10^6	1.02×10^4	99.947
2.4	1.7	0.49	29	36.4×10^6	4.2×10^3	99.988
3.5	0.24	0.31	31	22.5×10^6	3.9×10^4	99.83
3.5	0.24	0.32	31	20.8×10^6	1.4×10^3	99.993
3.5	0.95	0.42	31	26.0×10^6	2.8×10^4	99.89
3.5	0.95	0.41	31	27.1×10^6	1.1×10^4	99.959
3.5	0.45	0.38	1.5	39.0×10^6	5.6×10^3	99.986
3.5	0.45	0.40	1.5	34.5×10^6	6.4×10^4	99.81

*All tests used 1.0 kg/m² J-M 535 precoat and body feed, Johns-Manville, Denver, Colo.

TABLE 6
Granular media filtration with low coagulant doses

Length of Filter Run h	Alum Dose mg/L	Filtered Water Turbidity ntu	G. muris cysts/L: Raw Water	G. muris cysts/L: Filtered Water	Cysts Reduced percent	Test Conditions
Run 18						
0	2.2					raw water
0			8500			turbidity—4.5 ntu
0.5–1.5		0.58–0.51		1000	88	pH 6.5
2.5	2.1					temperature—20° C
3.0–4.0		0.63–0.46		1900	78-63	filtration rate—10 m/h
4.0	1.9					
4.8–5.7		0.79–0.77		4000	23	
5.7			5200			
Run 20						
0	1.4					raw water turbidity—4.4 ntu
0			5900			pH 6.5
0.9–1.4		1.0–0.95		1500	75	temperature—22° C
1.5	1.8					
2.3–2.7		0.65–0.60		420	94	filtration rate—10 m/h
2.8			7100			

Because data indicated that filter efficiency was related to precoat thickness, a series of tests was performed in which the filter was operated with precoat only. The tests were made with a single batch of water so that turbidity differences would not influence the results (Figure 3). Efficiency of microsphere removal increased with increasing precoat thickness up to 1.0 kg/m². Additional precoat did not improve filter performance.

Results from the membrane sampling filters showed that the microsphere removal capability of the DE filter usually improved during the run. The sampling filter was usually changed once during a filter run because of head loss buildup in the sampling filter. Counting data showed that the proportion of radioactive beads trapped during the first portion of the run almost always exceeded the proportion of the DE effluent that had passed through the filter. Conversely, the fraction of beads caught on the filter for the latter part of the run was almost always less than the proportion of effluent passing the sample filter in the latter part of the run. These data suggested that addition of body feed and removal of particulates produce a filter cake of increasing depth that is progressively more effective for particulate removal as the filter run continues.

Results of DE filtration experiments performed at 2.4 and 3.5 m/h show that *G. muris* cysts were very effectively removed at either filtration rate (Table 5). After the test with the lowest reduction of cyst concentration (99.36 percent) an above-normal amount of diatomite appeared in the concentrate from the centrifuge. Larger amounts of both diatomaceous earth and cysts passed through in this test. Although the septum had been cleaned before this test, it was cleaned again very carefully and examined for physical defects. None being found, the filter was reassembled and performed well thereafter.

Tests with cysts confirmed the findings with microspheres that turbidity removal is not a good indicator of cyst removal. Even when turbidity removal was low, cyst removal was high. This finding may be attributable to very small particles that could pass through the filter cake and cause a turbidity reading even though the larger cysts were strained out by the diatomite. High quality operation is the key to effective operation of DE filters.

Granular media filtration studies

Granular media filtration research emphasized assessment of the causes of poor and effective filter performance. Operating conditions were usually selected to achieve good cyst removal, but in some tests coagulant chemical doses were deliberately selected to give poor cyst removal. Filter operation was intention-

ally disturbed during some runs to show how this would affect cyst removal. One experimental goal was to determine whether changes in the cyst concentration of filtered water could be related to changes in filtered water turbidity.

Experimental methods. Jar tests were first performed in order to select appropriate doses of treatment chemicals and to make a preliminary assessment of the efficacy of coagulation and sedimentation for *Giardia muris* cyst removal. Combinations of treatment chemicals that were found effective were later tested in a pilot filter column. Alum, either alone or in combination with cationic polymer, was effective for coagulation of the cysts because of their negative zeta potential.

The filtration system (Figure 4) generally operated at 0.2 L/min with low-turbidity, gravel-pit water pumped through three inline mixers where cysts and coagulation chemicals were added, then through two or three 2-L enclosed jars with a 30-rpm stirrer in each for flocculation, and finally to a 3.8-cm diameter filter column with 46 cm of 1.27-mm effective size (e.s.) coal on top of 15 cm of 0.36-mm e.s. sand. Head loss through the filter column was measured with a mercury manometer. Filtered water was passed through an inline laboratory turbidimeter before discharge to waste or collection for analysis.

Samples to be analyzed for cysts were collected in 1-L cubitainers, refrigerated, concentrated, and counted by the method previously described.

Results. Test conditions included a broad range of possible field situations so that data on the ability of granular media filters to remove *G. muris* cysts could be developed. Alum coagulant doses ranged from none to adequate; cationic and nonionic polymers were also used. The effects of filter rate changes and loss of chemical feed were evaluated.

One filtration test was performed to determine the ability of a clean filter bed to remove uncoagulated cysts. Cysts were mixed into 67 L of unchlorinated, filtered well water at pH 8.2. The water was mixed and sampled for cysts, and the contaminated water containing 68 000 cysts/L was then pumped directly to the filter column for four hours. Cyst concentrations in filtered water ranged from 4100/L to 28 000/L in four samples, for removals of 94 to 59 percent.

Since those regions of the United States that have experienced *Giardia* problems tend to have clean, clear surface waters, filtration with minimal alum dose was also studied. Low coagulant doses are not unusual in locations where surface waters frequently have turbidities near or less than 5 ntu. The objective of the tests with low alum doses was to meet the 1-ntu maximum contaminant level (MCL) for turbidity, but not to produce the lowest possible turbidity. In these experiments the alum feed was changed during the filter run to vary the filtered water turbidity. Cyst samples were taken beginning about one half hour or longer after the change of coagulant dose, when filtered water turbidity was stable or declining. Results of these experiments are shown in Table 6. Higher filtered water turbidity was clearly associated with lower cyst removal efficiency, although the level of efficiency varied in the two runs.

Effective coagulation was attained in nine filter runs, resulting in filtered water turbidities ranging from 0.05 ntu to 0.30 ntu (generally greater than 0.2 ntu) during the portions of the runs that preceded turbidity increases or breakthrough. Cyst removal was good when filtered water turbidity was stable.

In six of the filter runs a typical ripening period occurred, and turbidity declined from above-normal to equilibrium values in approximately the first half hour of operation. In three such runs during the ripening phase, when turbidity was above normal, cysts were detected in concentrations substantially greater than those found later during equilibrium operating conditions. Initial cyst concentrations of 990, 690, and 910 cysts/L exceeded later values by factors of $\geq$ 25, 10, and 21, respectively, even though the turbidity changes observed during filter ripening were all decreases of less than 0.10 ntu.

Filtration rate increases during some filter runs caused effluent turbidity and cyst concentration to increase. Rate increases of 50, 100, or 150 percent in less than 10 s were studied to show the effect of sudden increases in flow at a treatment plant. A strong alum floc conditioned with nonionic polymer withstood the added shear at 20°C, but alum floc at 20°C and alum–nonionic polymer floc at 10°C broke through when flow rates increased.

During one filter run the rate was increased from 11 to 27 m/h for a duration of 2 min and then decreased again to 11 m/h. Turbidity rose sharply during this brief period of high rate operation, but it rapidly declined after the filtration rate was lowered. Cyst concentrations followed the trends in turbidity (Figure 5). When the rate of filtration was increased abruptly, a fourfold increase in turbidity was accompanied by a 25-fold increase in cyst concentration in filtered water.

Turbidity increases associated with loss of coagulant chemical feed also were associated with increases in the cyst counts. When chemical feed was interrupted during the run with 22 mg/L alum, cyst concentration rose from less than 80 to 790 and then to 1760 cysts/L as turbidity increased from 0.27 to 0.37 and

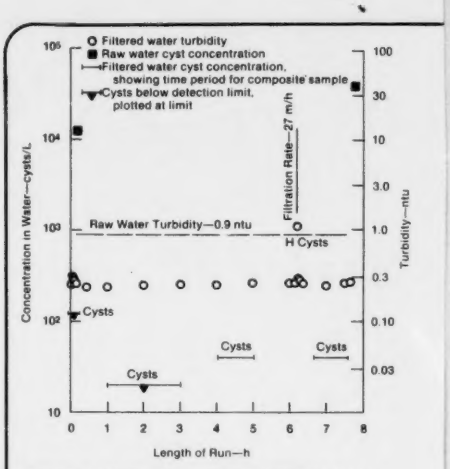

Figure 5. Operating data for filter run 11
Filtration rate 10 m/h except during evaluation of rate increase effects; pH 6.4; 9.8 mg/L alum; temperature 20° C.

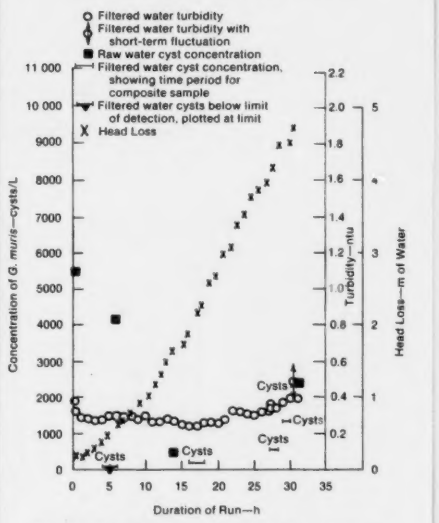
Filtered water turbidity
Filtered water turbidity with short-term fluctuation
Raw water cyst concentration
Filtered water cyst concentration, showing time period for composite sample
Filtered water cysts below limit of detection, plotted at limit
Head Loss
Concentration of G. muris—cysts/L
Turbidity—ntu
Head Loss—m of Water
Duration of Run—h

Figure 6. Operating data for filter run 15

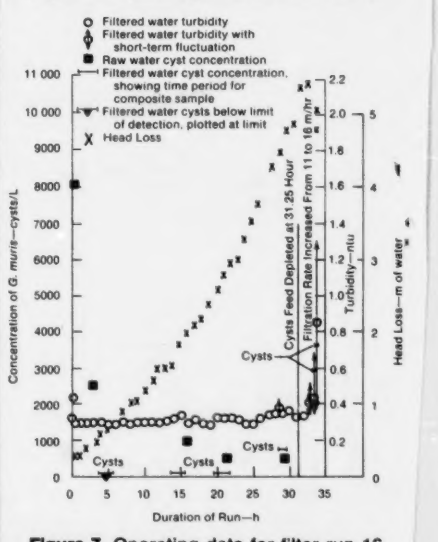
Filtered water turbidity
Filtered water turbidity with short-term fluctuation
Raw water cyst concentration
Filtered water cyst concentration, showing time period for composite sample
Filtered water cysts below limit of detection, plotted at limit
Head Loss
Cysts Feed Depleted at 31.25 Hour
Filtration Rate Increased From 11 to 16 m/hr
Concentration of G. muris—cysts/L
Turbidity—ntu
Head Loss—m of water
Duration of Run—h

Figure 7. Operating data for filter run 16

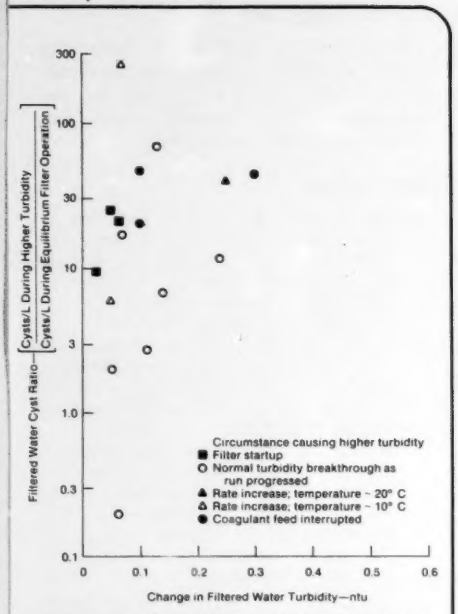

Figure 8. Relationship of cyst concentration changes to filtered water turbidity changes

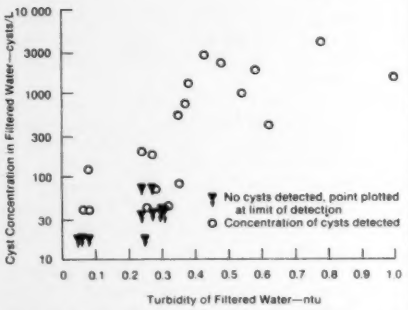

Figure 9. Filtered water turbidity versus cyst concentration for filtration of coagulated water at 10 m/h (constant rate)

TABLE 7
Summary of G. muris cyst removal during stable granular media filter operating conditions

Filtered Water Turbidity ntu	Cyst Concentration cysts/L		Removal percent
	Raw Water	Filtered Water	
0.27	14 000	< 40	> 99.71
0.27	16 000	< 80	> 99.50
0.27	45 000	180	99.60
0.28	61 000	70	99.88
0.24	5700	< 40	> 99.30
0.24	29 600	< 80	> 99.73
0.25	12 200	< 20	> 99.84
0.26	12 200	40	99.67
0.26	36 200	40	99.89
0.30	3200	< 40	> 98.7
0.24	490	200	59.2
0.30	2500	< 41	> 98.3
0.32	950	< 43	> 95.5
0.32	470	43	90.8
0.08	180 000	120	99.93
0.08	190 000	40	99.98
0.08	16 000	40	99.75
0.08	36 000	< 20	> 99.93
0.08	72 000	< 20	> 99.97
0.08	50 000	< 20	> 99.96

then to 0.57 ntu. When alum and cationic polymer feed were halted in another test, cyst concentration increased from 70 to 3300 cysts/L as turbidity rose from 0.28 to 0.38 ntu.

In order to evaluate the effects of turbidity breakthrough at the end of a longer filter run, two filter runs were continued for more than 24 hours. Graphs of head loss, turbidity, and cyst concentration are shown in Figures 6 and 7. Because the pilot column was installed as a pressure filter, the head loss in these runs exceeded the normal terminal head losses at which gravity filtration plants operate. Near the end of the two longer runs, slight turbidity increases were accompanied by large rises in the *G. muris* cyst concentrations.

In run 16 (Figure 7) filter operation was continued after the stock suspension of cysts being fed into the raw water was exhausted. Before breakthrough, turbidity was 0.30 ntu and 40 cysts/L were detected in the effluent. As the run continued, turbidity fluctuated between 0.35 and 0.4 ntu, and cyst count rose to 740 cysts/L. Two hours after the addition of cysts had ceased, filtered water turbidity varied between 0.36 and 0.7 ntu, and the cyst count of a sample taken at this time was 2900 cysts/L. This suggests that cysts removed in the filter migrated down through the media during the filter operation and were discharged along with floc sheared off the filter media.

Most of the research was done at water temperatures near 20°C but three of the runs were carried out at the low temperatures more typical of mountain streams or winter and spring conditions that have been associated with waterborne outbreaks of giardiasis. Raw water was chilled to 1°C. The flocculator jars were kept in an ice bath, but because the laboratory was at room temperature the water in the second (last) flocculator jar rose to 5° or 6°C. Even though the filter was insulated, water temperature rose to about 15°C at the filter outlet.

When the low temperature runs were made, raw water turbidity was very low, ranging from 0.15 to 0.20 ntu. Filtered water turbidity during the runs was 0.05 to 0.08 ntu. When abrupt rate increases occurred during these experiments, slight turbidity rises (to 0.10–0.17 ntu) and increases in cyst concentrations were observed.

Discussion of granular media results. The results of granular media filtration research indicate that a dual media filter operating in an equilibrium condition does remove a high percentage of *Giardia muris* cysts. Changes in the operation of a filter, such as a rate increase or a loss of chemical feed, can adversely affect effluent quality, causing pronounced deterioration. Cyst concentration increased when turbidity increased, but cyst counts rose more than turbidity.

Figure 8 shows the relationship between turbidity changes and cyst concentration changes. To emphasize the need for very careful control of filter effluent turbidity, the actual turbidity changes are shown. Because cyst counts varied from run to run, ratios of high to normal cyst counts are plotted. The ratios are minimum values because when no cysts were detected in filtered samples the detection limit was used as the denominator for the ratio. Most of the data represent increases of turbidity caused by breakthrough, rate increase, or coagulant feed loss plotted against the corresponding ratio of cysts at high turbidity to cysts at low turbidity. Three data points show the turbidity decrease during filter ripening plotted versus the cyst concentration ratio. In the filter ripening stage of treatment both turbidity and cyst concentration decreased.

The data showed one exception. During one of the longer runs the lowest observed turbidity of 0.24 ntu corresponded to a sample with 200 cysts/L, whereas the lowest cyst concentration, less than 40/L, occurred when turbidity averaged 0.30 ntu. Calculations for cyst and turbidity changes were based on the lowest observed turbidity in this run. This was the only observation of a decrease in turbidity accompanied by a rise in cysts.

The most significant aspect of the data in Figure 8 is that the equilibrium values of turbidity during the filter runs ranged from 0.05 ntu to 0.30 ntu, and in no case did the change in turbidity cause the filtered water turbidity to exceed the 1-ntu MCL.

The need for attaining filtered water turbidity at a level considerably below 1 ntu can also be seen in Figure 9, which shows cysts in filtered water plotted versus filtered water turbidity. Cyst counts as high as 4000 cysts/L were observed even though the 1-ntu MCL was being met. The data in Figure 9 were obtained from runs made with coagulated water at a rate of 10 to 11 m/h. These data suggest that turbidity should perhaps be as low as 0.3 ntu for effective cyst removal. Merely attaining the 1-ntu limit is not adequate for removal of *G. muris* cysts.

Because *G. lamblia* has caused a number of waterborne disease outbreaks, assessing the efficacy of granular media filtration for cyst removal was an important aspect of this research. In order to do this, Table 7 was prepared. It presents data taken from tests in which filter operation at 10 m/h with adequate coagulation produced water with a turbidity of 0.30 ntu or less.

Under the conditions described, cyst concentrations in filtered water ranged from less than 20 to 200 cysts/L. Only three of twenty samples had more than 100 cysts/L. As would be expected, the

highest removal percentage, 99.98 percent, was attained when the influent cyst concentration was highest, 190 000 cysts/L. During incidents of contamination when the cyst concentration in raw water is very high, properly operated granular media filters should remove a substantial portion of the cysts. In this research, when raw water cyst concentration exceeded 10 000 cysts/L, the filter always removed more than 99.5 percent of the cysts when operated properly.

On the other hand, two samples in Table 7 show that when cyst concentrations in flocculated water were lower (5700 cysts/L or less), some cysts passed through the filter. Cysts were not detected in four other samples. The continuous passage of a small number of cysts through the filter cannot be ruled out. Because of the detection limit problem inherent in microscopic enumeration of the cysts, in no case was the entire volume of concentrated filter effluent examined. Thus no data exist to show that all cysts were removed; i.e., the detection limit is always above zero.

At this time granular media filters cannot be considered a totally effective barrier against passage of *Giardia* cysts into drinking water. Another barrier, disinfection, must also be used. Disinfection research is still under way and definitive data are not available, but preliminary results suggest that substantially higher concentrations of chlorine are required to inactivate *Giardia* cysts as compared to coliforms. Chlorination practice that is barely adequate for inactivation of coliforms should not be expected to inactivate *Giardia* cysts.

Water utilities that are located in regions where giardiasis outbreaks have occurred and that rely on disinfection alone to treat low turbidity, colored surface waters face a particularly difficult situation. Use of very high doses of chlorine to control *Giardia* cysts could result in violation of the 0.10-mg/L MCL for trihalomethanes, a limit that is applicable to utilities serving 10 000 or more persons. Use of very low chlorine doses to control trihalomethanes might leave the water supply susceptible to a giardiasis outbreak. Water utility managers who find themselves in this situation should discuss the problem with regulatory officials and obtain advice on how to deal with it in the short term. The long-range solution would be installation and proper operation of a filtration plant.

Summary and conclusions

Diatomaceous earth research. Diatomaceous earth filtration research conducted since the early 1940s has shown that DE filters can effectively remove cysts and cyst-sized particles. Removal of particles in the 7 to 20-μm size range is not necessarily related to turbidity removal, as shown by both the US Army research and this work. Therefore, strict adherence to recommended operating technique is suggested for successful cyst removal. The following conclusions can be drawn:

- DE filtration for *Giardia* cyst removal is practical and effective as long as these filters are properly maintained and operated.
- Thickness of DE precoat had a greater effect on microsphere removal than did DE grade (particle size).
- A precoat of 1.0 kg/m² provided an effective barrier to cyst passage.
- DE filter efficiency for microsphere removal generally increased as the filter runs progressed.
- DE filters used in the production of potable water should not be operated without body feed addition.
- Because effluent turbidity is not an effective indicator of a DE filter's ability to remove *Giardia* cysts, reliance must be placed instead on use of proper treatment techniques with an adequate amount of DE precoat and body feed.

Granular media filtration tests. This work represents initial efforts in the investigation of filtration techniques for removal of *Giardia* cysts from drinking water. As only limited data are available at this time, it would be premature to suggest a filtered water turbidity goal that would assure successful cyst removal. Although pilot plant work done at the University of Washington through 1979 generally confirmed earlier EPA results, further research is needed before a turbidity goal can be recommended. On the basis of this early work, however, some conclusions can be formed.

- Meeting the 1-ntu MCL for turbidity did not result in effective cyst removal.
- Increases in filtered water turbidity, even as low as 0.05 to 0.10 ntu, were usually associated with large increases in cyst concentrations.
- Cyst concentrations during the filter ripening period are likely to be higher than they are after turbidity has stabilized. One way to avoid problems of higher cyst concentrations at this time would be to filter to waste for perhaps the first half hour of a filter run.
- After a stable effluent turbidity has been achieved, increases of turbidity should be avoided in order to minimize passage of *Giardia* cysts through the filter.
- Although a granular media filter should not be expected to remove all cysts from the raw water, a filter treating water dosed with an adequate amount of coagulant chemical and operated in a manner that prevents filtered water turbidity increases should remove a very substantial portion of the *Giardia* cysts.
- The multiple barrier concept should be employed to treat water likely to contain *Giardia* cysts. Use of both filtration and disinfection is necessary.

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